PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 030331wo/Me/sto	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/04872	International filing date *(day/month/year)* 09.05.2003	Priority date *(day/month/year)* 10.05.2002
International Patent Classification (IPC) or both national classification and IPC A61K38/19		
Applicant IPF PHARMACEUTICALS GMBH et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
10.12.2003	14.09.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Lechner, O Telephone No. +49 89 2399-8687

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/04872

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-21, 23-33	as originally filed
22	filed with the demand

Claims, Numbers

1-18	filed with the demand

Drawings, Sheets

1/12-12/12	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 1-18 (only partially)

   because:

   ☒ the said international application, or the said claims Nos. 1-13 (concerning industral applicability) relate to the following subject matter which does not require an international preliminary examination (specify):

   see separate sheet

   ☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 9 (partially) are so unclear that no meaningful opinion could be formed *(specify)*:

   see separate sheet

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1-18 (all in part)

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-18 (all in part) .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-10, 13
	No:	Claims	11, 12, 14-18
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-18
Industrial applicability (IA)	Yes:	Claims	
	No:	Claims	c.f. SepSheet

2. Citations and explanations

   see separate sheet

Item I

Amended **claims 1-18** and page 22 filed with the demand dated 10.12.2003 do not introduce subject-matter which extends beyond the content of the application as filed and thus are considered to be allowable in the sense of **Article 34(2)(b) PCT**.

Item III

1. **Non-establishment of opinion with regard to novelty, inventive step and industrial applicability (Rule 67.1, PCT)**
   Claims 1-13 relate to subject matter considered by this Authority to be covered by the provisions of **Rule 67.1(iv), PCT**. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject matter of these claims **(Article 34(4)(a)(I), PCT)**.

2. The present IPER was limited to those parts which have been searched, i.e. the use of R-CCL14[10-74] (R as defined in claims 7-10) for inhibiting the emigration of cells from the intravascular compartment into tissues (in vitro) and for treating inflammatory and other specific diseases (in vivo) mentioned on page 7, line 10-15 (allergic asthma - benign prostatic hypertrophy) **(Rule 66.1(e), PCT)**.

Item IV

1 Reference is made to the following document:

D1 EP-A-1 167 527 (EUROSCREEN S A ;NIEDERSAECHSISCHES INST FUER P (DE)) 2 January 2002 (2002-01-02)

D2 MUENCH JAN ET AL: 'Hemofiltrate CC chemokine 1(9-74) causes effective internalization of CCR5 and is a potent inhibitor of R5-tropic human immunodeficiency virus type 1 strains in primary T cells and macrophages.' ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol. 46, no. 4, April 2002 (2002-04), pages 982-990, XP002241992 April, 2002 ISSN: 0066- 4804

2 Non Unity (Rule 13(1), PCT)

The Examining Division agrees with the objection put forward by the Search Division as to lack of unity, the reasons of the objection being as follows:

The problem to be solved by the present invention is to provide methods for inhibiting the emigration of cells from the intravascular compartment into tissues.
The use of a chemoattractant for inhibiting the emigration of cells from the intravascular compartment into tissues represents the technical feature which may a priori be regarded as the single general concept involved in the technical relationship among the

different inventions listed above.

However, **D1** discloses the use of CCL14 [9-74] and CCL14 [12-74] for treating various diseases such as inflammation by influencing migration of leukocytes. Accordingly, the peptides may be modified by attaching various hydrophobic or polar aprotic residues with organic residues with up to 50 amino acids, any amino acid or CH3-(CH2)n-X, whereby n = 1-17 and X= e.g. -C(O)-NH-CH2-C(O) (abstract; §15, 41, 61; claims 1-6, 20-22). I.e. **D1** employs molecules covered by the general formula R*-CCL14[10-74] for treating various diseases such as inflammation by influencing migration of leukocytes (abstract; § 15, 41, 61; claims 1-6, 20-22).
Consequently, the idea of using a chemoattractant for inhibiting the emigration of cells from the intravascular compartment into tissues is already known in the state of the art and can, therefore, not serve as a single general inventive concept linking the 5 proposed technical solutions:

1) use of R*-CCL14[10-74];
2) use of R*-CXCL12[1-72] and functional variants thereof
3) use of a defensin
4) use of a leukotriene
5) use of a formyl-peptide

In the present application no further technical feature(s) can be distinguished that can be regarded as the common inventive concept involved in the technical relationship among the five different inventions.
Consequently, the present application lacks unity of invention in the sense of **Rule 13(1), PCT**, and the different solutions not belonging to a common inventive concept are identified as the different inventions listed below. Each of the inventions listed is a distinct invention, characterised by its own special technical feature, defining the contribution which each of the claimed inventions, considered as a whole, makes over the prior art.
Hence, the International Examining Authority considers that the following separate inventions of groups of inventions are not linked a to form a general inventive concept:

R*...as defined in claims 7-10; **...all partially

#	claims	problem to be solved	solution
1	1-18**	provide a method for inhibiting the emigration of cells from the intravascular compartment into tissues	use of R*-CCL14[10-74]
2	1-17**	-"-	use of R*-CXCL12[1-72] and functional variants thereof
3	1-6, 13-16**	-"-	use of a defensin
4	1-6, 13-16**	-"-	use of a leukotriene
5	1-6, 13-16**	-"-	use of a formyl-peptide

Item V

Upon reconsideration of the case in view of the arguments provided by the applicant in his letter of 11.05.2004, the IPEA came to the following conclusion:

1 Novelty (Article 33(2), PCT)

1.1 **D1** discloses the use of CCL14[9-74] and CCL14[12-74] for treating and diagnosing various diseases such as inflammation (incl. rheumatoid arthritis), allergies (incl. asthma), infection (e.g. HIV) and others by influencing migration of leukocytes (c.f. claim 1-6, 20-23; §0041; §0061). The peptides according to **D1** affect the chemotaxis/migration of eosinophils, T-lymphocytes, monocytes and dendritic cells. Accordingly, the peptides may be modified by attaching various hydrophobic or polar aprotic residues, organic residues, polyethyleneglycol and any amino acid or CH3-(CH2)n-X, whereby n = 2-9 and X= e.g. -C(O)-NH-CH2-C(O) (c.f. §0010, §0013, §0015; §0017, §0061). Specific modifications proposed in **D1** are the N-terminal attachment of a pentane oxime or nonanoyl(NNY)-residue (c.f. §0015). Example 6 in **D1** shows that CCL14[9-74] inhibits HIV-1 entry/replication in human cells (§0097). Thus, in view of the teaching of **D1**, the subject matter of **claims 11, 12, 14-18**, as far as examined (c.f. point III.2), would not appear to be novel in the sense of **Article 33(2), PCT.**

Remark: If the use of a medicament for treating a specific pathology/disease (e.g. HIV-1 infection and HIV-related inflammation) is already known from the prior art, subject matter relating to specific mechanisms of action of the medicament is considered to be already implicitly disclosed by the prior art.

1.2 No documents are comprised in the known prior art explicitly disclosing a method of

inhibiting the emigration of cells from the intravascular compartment into tissues or through any membrane limiting any body compartment from another in vitro by confronting the cells with R-CCL14[10-74] (R as defined in claims 7-10), thereby making the cells unresponsive to further activation.
Also no documents are comprised in the known prior art explicitly disclosing the use of R-CCL14[10-74] (R as defined in claims 7-10) for treating the specific inflammatory diseases allergic asthma, atopic dermatitis and rheumatoid arthritis.
Thus, the subject matter of **claims 1-10** and **13**, as far as examined (c.f. item III.2), would appear to be novel in the sense of **Article 33(2), PCT**.

2 Inventive step (Article 33(3), PCT)

D1 discloses the use of CCL14[9-74] and CCL14[12-74] for treating and diagnosing various diseases such as inflammation (incl. rheumatoid arthritis), allergies (incl. asthma), infection (e.g. HIV) and others by influencing migration of leukocytes (c.f. claim 1-6, 20-23; §0041; §0061). The peptides according to **D1** affect the chemotaxis/migration of eosinophils, T-lymphocytes, monocytes and dendritic cells. Accordingly, the peptides may be modified by attaching various hydrophobic or polar aprotic residues, organic residues, polyethyleneglycol and any amino acid or CH3-(CH2)n-X, whereby n = 2-9 and X= e.g. -C(O)-NH-CH2-C(O) (c.f. §0010, §0013, §0015; §0017, §0061). Specific modifications proposed in **D1** are the N-terminal attachment of a pentane oxime or nonanoyl(NNY)-residue (c.f. §0015). Example 6 in **D1** shows that CCL14[9-74] inhibits HIV-1 entry/replication in human cells (§0097).
The examples provided in **D1** show that CCL14[9-74] is a potent inhibitor of HIV replication (c.f. §0097).

2.1 The subject matter of **claim 1**, as far as examined (c.f. item III.2), differs from **D1** in that it claims a method of inhibiting the emigration of cells from the intravascular compartment into tissues or through any membrane limiting any body compartment from another in vitro by confronting the cells with R-CCL14[10-74] (R as defined in claims 7-10), thereby making the cells unresponsive to further activation.
The problem to be solved is to provide a method of inhibiting the emigration of cells from the intravascular compartment into tissues or through any membrane limiting any body compartment from another in vitro.
The claimed solution, as far as examined (c.f. point III of the second written opinion), is a method using R-CCL14[10-74] (R...as defined in claims 7- 10).

In contrast to the interpretation given by the applicant in his letter of 11.05.2004, the IPEA is of the opinion, that the subject matter of **claim 1,** as far as examined (c.f. point

III of the second written opinion) would be obvious for a skilled person in view of the closest prior art document **D1** for the following reasons:
Taking into consideration the experimental data provided in **D1,** i.e. preincubation with CCL14[9-74] leading to total desensitization of monocytes, abrogating further responses to RANTES (§0095, line 30-35), it would be obvious for the skilled person, that he could use a preincubation step with CCL14[9-74] to render cells unresponsive to e.g. RANTES-mediated effects such as extravasation in vitro.
Combining the teachings of **D1** and **D2** (CCL14[10-74] and [9-74] show same activity; CCL14[9-74] analogues modified in analogy to modifications described for RANTES showing higher activity) the skilled person would have a clear incentive to test also CCL14[10-74] analogues in order to obtain alternative CCL14 molecules with increased activity which can be used to inhibit the effects of RANTES in vitro e.g. extravasation and would, thus, have arrived with a high expectation of success to the specific molecules and methods covered by present **claim 1.**
Therefore, the subject matter of **claim 1** and, consequently, also of **claims 2-10,** all as far as examined (c.f. point III.2), would not appear to involve an inventive step in the sense of **Article 33(3), PCT.**

2.2 The subject matter of **claim 13**, as far as examined (c.f. item III.2), differs from **D1** in that it claims the use of R-CCL14[10-74] (R as defined in claims 7-10) for treating asthma, atopic dermatitis and rheumatoid arthritis.
The problem to be solved is to provide (a) medicament(s) for treating allergic asthma, atopic dermatitis and rheumatoid arthritis.
The claimed solution, as far as examined (c.f. point III.2), is the use of R-CCL14[10-74](R as defined in claims 7-10).

Although claim 22 of **D1** claims the treatment of rheumatoid arthritis, allergies, asthma etc., it is not clear (apart from the clearcut data for HIV-1 provided in example 6) from claim 22 and the whole document, respectively, if CCL14[9-74] or an antagonist thereof should be used as a medicament for treating the claimed diseases/pathologies.
Moreover, the present application provides evidence, that, NNY-CCL14[10-74] is able to significantly reduce the influx of eosinophils into airways in vivo in a murine model of allergic asthma also when applied 3h or 8h after allergen aerosol provocation.
Therefore, the subject matter relating to the use of NNY-CCL14[10-74] for treating the specific inflammatory disorders mentioned above, would appear to be inventive.

Notwithstanding (as already mentioned in the second written opinion), the subject

matter of present **claim 13**, as far as examined (c.f. point III.2), also covers R-CCL-14[10-74] molecules such as e.g. Bis-NNY-, which behave in a different way compared to NNY-CCL-14[10-74]: The description states that ten different CCL14 analogues have been tested: NNY-CCL14[10-74], Bis-NNY-CCL14[10-74], CCL14[1-74]/[6-74]/[7-74]/[8-74]/[9-74]/[10-74]/[11-74] and [12-74] (c.f. p 14, l 3-8).
From these ten CCL14 molecules, apparently, only CCL14[8-74], CCL14[9-74], CCL14[10-74] and NNY-CCL14[10-74] are able to induce a significant release of ROS at concentrations up to 10e-7M (c.f. p 14, l 18-22; Fig 2), while Bis-NNY-CCL14[10-74] is not. From this four molecules, only CCL14[9-74], CCL14[10-74] and NNY-CCL14[10-74] are stated to be able to induce CCR downregulation, while the other derivatives, including Bis-NNY-CCL14[10-74] are not.

The applicant argues in his letter of 11.05.2004, that it can not be excluded that N-terminal modifications other than NNY- exert the same effects as NNY-CCL14[10-74] on the emigration of cells via other mechanisms such as receptor desensitisation and others.
However, due to the obvious unpredictable effect(s) of minor modifications at the amino-terminus of CCL-14[10-74] on its activities, it is also unpredictable, if all R-CCL14[10-74] molecules covered by present **claim 13** would be able to solve the technical problem, i.e. to treat one of the specific inflammatory disorders mentioned above.
Thus, it is not possible to acknowledge the presence of an inventive in the sense of **Article 33(3), PCT** for the whole width of present **claim 13**.

3 further remarks

3.1 Contrary to the requirements of **Rule 5.1(a)(ii), PCT**, the relevant background art disclosed in **D1**, is not mentioned/discussed in the description, nor is this document identified therein.

3.2 The vague statements in the description such as on page 3, line 1-7 or page 9, line 24-25 imply that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity (**Article 6 PCT**) when used to interpret them (see also the **PCT Guidelines, III-4.3a**).

Abbreviations

CCL, CC chemokine ligand; CRIC3, n-nonanoyl-CCL14[*10-74*]; bis-NNY-CCL14[*10-74*], Bis-n-nonanoyl-CCL14[*10-74*]; RANTES, regulation upon activation and T cell secreted; BALF, bronchoalveolar lavage fluid; AHR, airway hyper responsiveness; OVA, ovalbumin

Fig. 1:

Alignment of N-terminal sequences of CCL14 derivatives and CCL11.

The cleavage motif for CD26/DPP IV of CCL14[9-74] and CCL11 (eotaxin) is marked in gray.

Fig. 2:

CRIC3 induces the release of reactive oxygen species (ROS) from human eosinophils with more potency than CCL11.

The release of ROS was measured using lucigenin-dependent chemiluminescence. Human eosinophils were stimulated with different concentrations of the indicated chemokine. Data (n = 7) are expressed as relative ROS release that is expressed as the ratio of stimulus-stimulated and medium-stimulated cells.

Fig. 3:

CRIC3 induces an internalization of CCR3 from human eosinophils in the same range than CCL11.

Human eosinophils were treated with the indicated CCL14 derivatives (10^{-7} M) and CCL11(10^{-7} M), respectively, for 30 min at 37°C. Thereafter cells were stained with anti-CCR3 mAb and analyzed by flow cytometry. A: Data (n=4) are expressed as the mean ± SEM of relative fluorescence intensity as described in *Materials and Methods*. B: Histogram analysis of one representative experiment shown in Fig. A. Bold line, anti-CCR3 staining before chemokine treatment; dotted line, isotype control; broken line, anti-CCR3 staining after chemokine treatment. C: Cells were incubated with the indicated

Claims

1. A method of inhibiting the emigration of cells from the intravascular compartment into tissues (or through any membrane limiting any body compartment from another) by confronting the cells with an agonist specific for receptors involved with migration of said cells via a receptor thereby making the cell unresponsive to further activation.

2. A method according to claim 1, wherein the cells are blood circulating cells and the intravascular compartiment is the blood stream.

3. The method of claim 1 wherein the cells are leukocytes.

4. The method of claim 1 or 3 wherein the cell is unresponsive to further activation for emigration to tissues after confrontation with an agonist.

5. The method according to claims 1 to 4 wherein the agonist used to inhibit the migration of the cells is a chemoattractant binding to a corresponding receptor or molecule binding to such a receptor.

6. The method of claim 5 wherein the chemo-attractant is selected from the group consisting of chemokine, a defensine, a leukotriene, a formyl-peptide or combinations thereof as well as mutants and/or variants of the chemoattractant.

7. The method of claim 1 to 6 wherein the compound is selected from the group consisting of

   R^1-CCL14[10-74], R1-CXCL12[1-67], R1-CXCL12V3I[1-67], R1-CXCL12[2-67], R1-CXCL12V3I[2-67], R1-CXCL12[1-72], R1-CXCL12V3I[1-72], R1-CXCL12[2-72] and R1-CXCL12V3I[2-72]

   wherein R^1 is a lipophilic, hydrophobic or polar aprotic residue.

8. The method of at least one of the claims 1 to 7, wherein R^1 is any organic residue having up to 50 carbon atoms, which may be substituted by hetero atoms, and which organic residue is branched, unbranched, saturated, unsaturated or combinations thereof.

9. The method of claim 8, wherein R^1 is an aromatic moiety, polyethylenoxid, moiety with 2 to 18 units, comprising residue.

10. The method of claim 7, wherein R^1 is any amino acid, or CH_3-$(CH_2)_n$-X; in which

$(CH_2)_n$ is branched or unbranched

X is -C(O)-NH-CH_2-C(O)-, -NHCH_2-C(O)-, -ONH-CH_2-C(O)-,

-OCH_2-CH_2-C(O)-, -CH=CH-C(O)-, -C(O)-, or a covalent bond; and n is an integer of 1-17;

or pharmaceutically acceptable salt thereof.

11. A method of treating a disease state in mammals that is alleviated by treatment with a compound of at least one of the claims 7 to 10, which method comprises administering to an mammal in need of such a treatment a therapeutically effective amount of the compound.

12. The method of claim 5 wherein said method inhibits inflammation.

13. The method of claim 12, wherein inflammation is selected from the group consisting of allergic asthma, atopic dermatitis, rheumatoid arthritis, and combinations thereof.

14. Use of an agonist specific for receptor involved with migration of blood circulating cells from the blood stream for the manufacturing of a medicament for the treatment of diseases associated with migration of blood cells from the blood stream into tissues.

15. Use according to claim 14 wherein the agonist is a chemo-attractant.

16. Use according to claim 14 wherein the chemo-attractant is selected from the group consisting of chemokine, defensin, leukotriene, formyl-peptides as well as mutants and/or variants of the chemo-attractants.

17. Use of a compound of the method of at least one of the claims 7 to 10 for the manufacturing of a medicament for the treatment of diseases associated with migration of blood cells from the blood stream into tissues.